ARTHRODERMA SPECIES FROM THE „BARADLA" CAVE IN AGGTELEK (Biospeologica Hungarica, XXXI.)

by

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The isolation of dermatophytes from the soil has started in Hungary in the middle of the fifties. The first significant results were published by J. Bánhegyi in 1959. Among the species described *Keratinomyces ajelloi* Vanbr. was found only in the "Baradla" stalactite cave in Aggtelek and was reported for the first time to occur in Hungary.

Subsequently systematic mycological studies were started in order to describe the microflora of the cave with a special emphasis on the keratin-decomposing fungi. As a result of these studies the author has published the occurrence of the following species: *Myxotrichum chartarum* (Nees) Kunze, *Gymnoascus reessii* Baranetzky, *Trichophyton terrestre* Durie et Frey, *Arthroderma quadrifidum* Dawson et Gentles (1962); *Myxotrichum deflexum* Berkeley, *Ctenomyces serratus* Eidam, *Microsporon gypseum* (Bodin) Guiart et Grigorakis, *Anixiopsis stercoraria* Hansen, *Pseudeurotium zonatum* van Beyma, *Chrysosporium merdarium* (Link) Carmichael, *Chrysosporium pannorum* (Link) Hughes (1966); *Chrysosporium keratinophilum* (Frey) Carmichael and *Chrysosporium evolceanui* (Rhandhawa et Sandhu) Garg (1968). In the present paper the isolation of the species *Arthroderma uncinatum* Dawson et Gentles, *Arthroderma curreyi* Berkeley and *Arthroderma tuberculatum* Kuehn is reported.

Materials and methods

The soil and bat guano was collected with sterile instruments from different parts of the big stalactite cave located in north-eastern in Hungary (in the county of Borsod). The list of the sampling areas and the dates can be found with the description of the individual species. An upper layer of 5 cm of the soil was discarded.

The isolation was, in this case, too, performed with the To-Ka-Va hair-baiting method which was most successfully applied. The baits were autoclaved fair and brown children hair, hen feather and horsehair. Depending on

the quantity of the material two or three parallels were used with the same bait. Where the material was scarce, the bait was varied in the hope that the specimens decomposing keratin will have a broader spectrum. The cultures were incubated at 28°C in Petri dishes and the humidity was maintained with sterile distilled water.

The full-grown fungal colonies were transferred onto Sabouraud glucose agar plates containing Acti-dione, penicillin and streptomycin. The pure cultures obtained were kept on Sabouraud glucose slants. Considering our observation that the formation of fruiting bodies on culture media are rare and even in this case they develop in a small number – the cultures were tested occasionally and in each case successfully by transferring the stock cultures onto sterile soil containing the relevant bait.

It is worth mentioning that the designations in the text with quotation marks represent the names of the different galleries or dripstones (stalactite/stalagmite) of the cave and are essential for the localisation of the place of sampling.

Results

Arthroderma uncinatum Dawson et Gentles, 1961

The imperfect form of the species, the *Keratinomyces ajelloi* was isolated from the cave several times. For the first time it outgrew from soil mixed with bat guano collected from untrodden soil that is 300 metres away from the entrance of the cave (Bánhegyi 1959). Hereupon we were not able to demonstrate the species either from soil or from guano for a long time. Next we succeeded in isolating from the muddy clay of the pond of the cave the bottom of which was accessible owing to the low water level in March and June, 1966. On the next occasion the imperfect form outgrew from argillaceous debris collected near the "Petőfi koporsója" (Petőfi's Coffin) in the hall of the "Paradicsom" (Paradise) in October, 1966.

Finally, the perfect form of the fungus was isolated from a muddy sample collected from the bottom of the lake surrounding the monumental dripstone called „Nádoroszlop" (Palatine Column) on the 28th of December, 1967 and the growth of which was observed on horsehair bait on the 2nd of February, 1968. (Table I.)

It is noteworthy that besides the imperfect form we found the fruiting body of *Anixiopsis stercoraria* on the samples of Agg. G 17-a, Agg. G 24-a and Agg. G 60-d. The first was grown on feather the others on brown children hair. Benedek (1963) considers the species of *Keratinomyces ajelloi* as an imperfect form of two Ascomyces. The author, in a work published in 1966, investigated the species of *Anixiopsis stercoraria* by means of a series of hanging drop cultures. Familiar with the relevant paper of Benedek, special attention was paid to the kind of conidia developed in the hanging drop cultures. In our experiments no macroconidia of *Keratinomyces* could be found. Neither perithecia nor macroconidia of *Anixiopsis stercoraria* were found in cultures of several *Keratinomyces ajelloi* strains. Thus our observation on strains isolated from soil do not support the results of Benedek obtained with the isolates from infections.

Table I.

Strain symbol	Bait	Place of sampling	Sample	Date of sampling	Number of the parallels
Agg. G 17 – a	feather	Palatine Column	muddy clay	23. 3. 1966	6
– b	hair				
Agg. G 23 – a	hair	Riverside	muddy clay	15. 6. 1966	10
– c	feather				
– e	horsehair				
Agg. G 24 – a	hair	Palatine Column	muddy clay	15. 6. 1966	9
– d	feather				
– e	horsehair				
– f	horsehair				
Agg. G 27 – c	horsehair	Paradise, Petőfi's Coffin	argillaceous debris	21. 10. 1966	3
Agg. G 59 – a	feather	Palatine Column	muddy clay	18. 12. 1967	6
– e*	horsehair				
– f	horsehair				
Agg. G 60 – a	feather				
– d	hair	Riverside	muddy clay	18. 12. 1967	6
– e	horsehair				
– f	horsehair				

* Perfect form

Cleistothecia are globose, white when young, light-brown when matured, the diameters being 250 – 1000 μ with appendages. The thickness of the peridial layer is 70 – 150 μ in proportion to the size of the cleistothecia. The distinction between the peridial hyphae and the central parts which are more heavily coloured and contain the spores, is not easy. The peridial hyphae are composed of regular dumb-bell shaped cells with rough walls, 6.4 – 12.0 × 2.0 – 6.4 μ. In addition to spiral appendages common with the genus Arthroderma there are multicellular, smooth, thick-walled, spindlelike macroconidia on the cleistothecia which are identical with the macroconidia of the imperfect form of *Keratinomyces ajelloi*. The size of the macroconidia is 24.0 – 83.0 × 2.0 – 10.5 μ. The asci are globose, subglobose, thin evanescent-walled, 4.8 – 7·2 × 4.8 – 6.4 μ with 8 spores. Ascospores are lenticular, yellow in mass, 2.4 – 2.8 × 4.0 – 1.6 μ.

Arthroderma tuberculatum K u e h n 1960

The species was described by K u e h n in 1960 as the second representative of the genus. He has stated that the species highly resembles the *Arthroderma curreyi* in the shape of the cleistothecium but obviously different with respect to the aleuriospores.

The occurrence of the species has not been reported from Hungary. We succeeded in isolating it from the hall of the "Paradicsom" (Paradise) only. First it outgrew from argillaceous debris sample collected near the "Petőfi koporsója" (Petőfi's Coffin) – tumbled, black stalagmite. It was possible to collect from the same point material sufficient for only 3 parallel samples

half a year later but fortunately it appeared on one of the samples. We were able to demonstrate the occurrence of the species from bat guano collected of another part of the hall, too. (Table II.)

Table II.

Strain symbol	Bait	Place of sampling	Sample	Date of sampling	Number of the parallels
Agg. G 15 – a	feather	Petőfi's Coffin	argillaceous debris	23. 3. 1966	5
– b	hair				
Agg. G 27 – a	hair	Petőfi's Coffin	argillaceous debris	21.10. 1966	3
Agg. G 28 – a	hair	Paradise, right passage	bat guano	21. 10. 1966	6
– b	hair				
– c	feather				
– d	feather				
– e	horsehair				
– f	horsehair				
Agg. G 55 – a	feather	Paradise, right passage	bat guano	18. 12. 1967	7
– b	feather				
– c	feather				
– f	horsehair				
– g	horsehair				

For the identification of the species the media described by K u e h n was used. During cultivation the same results with those described were obtained.

Cleistothecia are white when young, they become yellow-drab when mature. Their size with the appendages is $390-960\ \mu$. The thickness of the peridial hyphae is $130-320\ \mu$. This represents about one third of the full-sized cleistothecium. The ramose peridial hyphae are composed of dumb-bell shaped, rough cells with symmetrical and asymmetrical forms, $10-30\times4-7\ \mu$. The peridial hyphae have many slightly curved free ends bearing a few spiral appendages. Asci are ovoid, hyaline, evanescent, $4.0-4.8\times4.4-5.6\ \mu$ with 8 spores. Ascospores are smooth, yellow, flattened, oblate $1.2-2.0\times2.4-3.2\ \mu$. The imperfect stage is represented by cream-coloured, subglobose aleuriospores, $8.8-14.0\times13.6-20.0\ \mu$. As a rule they always form singly on short pedicellate or sessile, smooth when young, tuberculate or asperulate when matured. They occur in a great number.

Arthroderma curreyi B e r k e l e y 1860

We succeeded in isolating the species five times, the single representative of the genus for hundred years, from different parts of the cave. (Table III.)

On the basis of the characteristic form and size of the aleuriospores of *Arthroderma tuberculatum* we were able to identify easily *Arthroderma curreyi*.

This homothallic species always contains cleistothecia on bat guano in a great number.

Table III.

Strain symbol	Bait	Place of sampling	Sample	Date of sampling	Number of the parallels
Agg. G 26 – a	hair	Laboratory, big rock	bat guano	21. 10. 1966	9
– b	hair				
Agg. G 29 – j	feather	Laboratory, small rock	bat guano	21. 10. 1966	13
Agg. G 30 – a	hair	Bat gallery	bat guano	23. 2. 1967	8
– b	hair				
– f	horsehair				
Agg. G 36 – j	hair	Fox hole, flue	bat guano	13. 4. 1967	12
Agg. G 49 – a	feather	Laboratory, big rock	bat guano	22. 9. 1967	6
Agg. G 50 – a	feather	Laboratory, big rock	bat guano	18. 12. 1867	5
– b	feather				
– c	hair				
– d	hair				
– e	horsehair				

Cleistothecia are light creamy coloured, globose, subglobose, their size is 250 – 600 μ rarely exceeding the upper limit. The peridial hyphae are composed of rough symmetrical and asymmetrical dumb-bell shaped cells with a number of free ends having some spiral appendages. Asci are evanescent, globose or subglobose, $4.0 - 5.0 \times 5.0 - 6.4\ \mu$ with 8 spores. Ascospores are smooth, hyaline, lenticular, $2.4 - 3.2 \times 1.2 - 2.0\ \mu$. The imperfect stage is represented by smooth, clavate, single aleuriospores, sessile or developed on short pedicellate. They are to be found in a great number, $1.6 - 2.0 \times 2.8 - 4.8\ \mu$.

Summary

Further results of mycological examinations carried out in the "Baradla" stalactite cave in Aggtelek concerning keratin decomposing fungi are reported. The author succeeded in isolating with the To-Ka-Va keratin hairbait method *Arthroderma uncinatum* Dawson et Gentles 1961, *Arthroderma tuberculatum* Kuehn 1960 and *Arthroderma curreyi* Berkeley 1860. All 3 species are reported for the first time to occur in soil or bat guano in Hungary.

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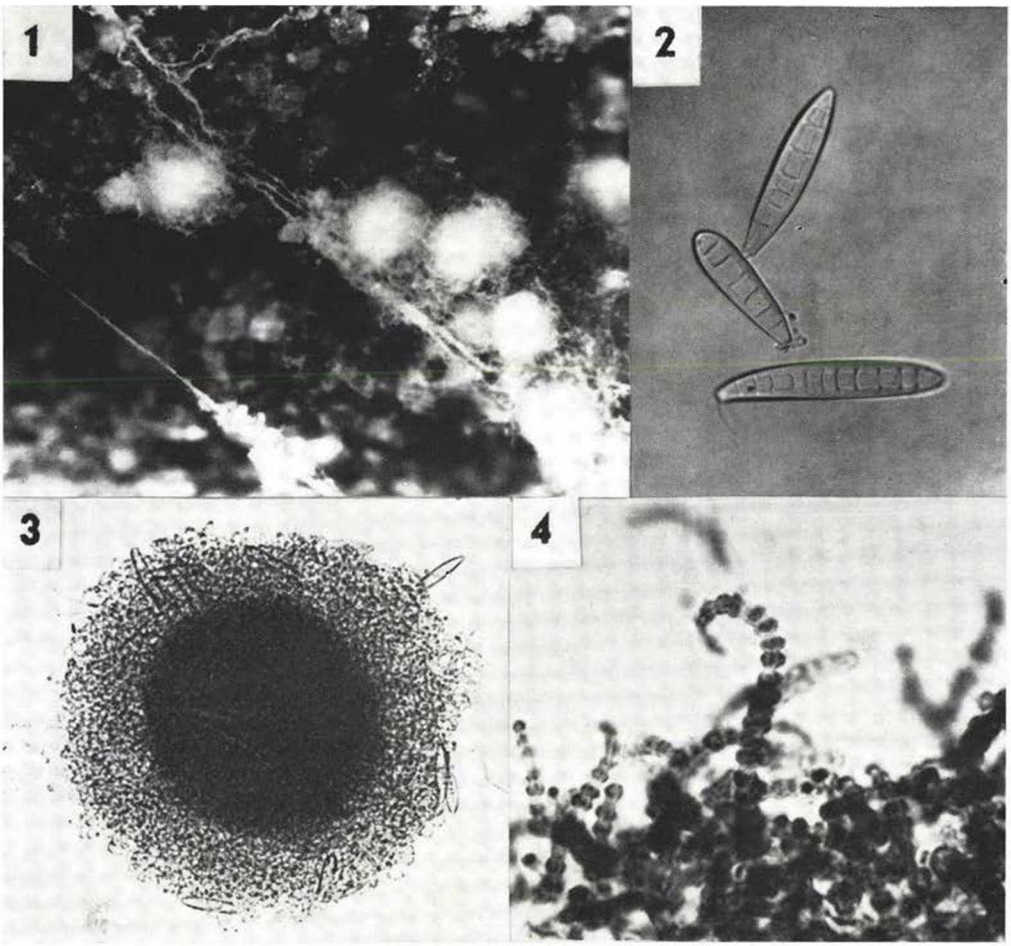

Fig. 1. *Arthroderma uncinatum*. 1 – Mature cleistothecia on horsehair bait × 18; 2 – The imperfect form *(Keratinomyces ajelloi)* macroconidia × 400; 3 – Mature cleistothecium × 85; 4 – Peridial hyphae showing dumb-bell shaped cells × 500.

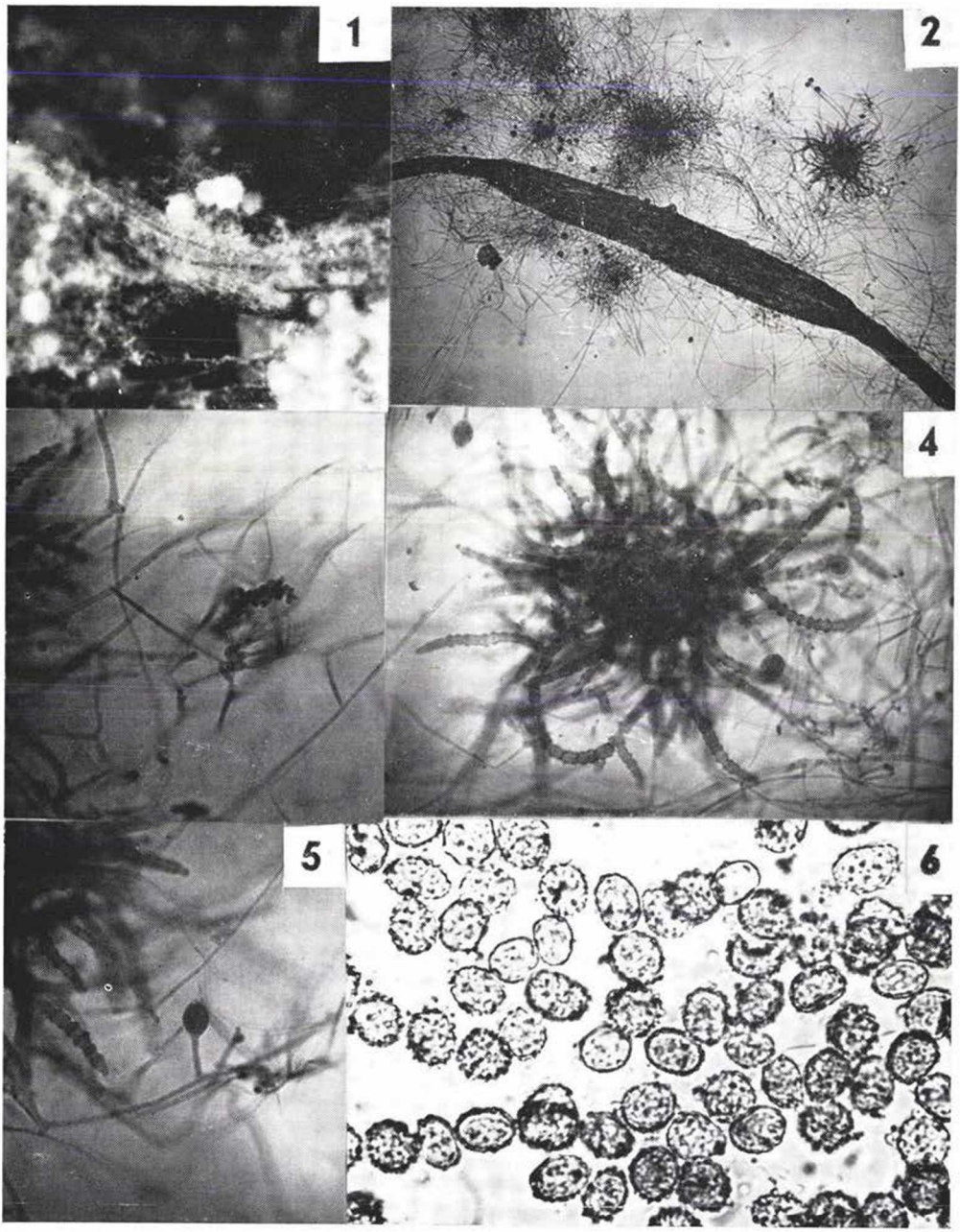

Fig. 2. *Arthroderma tuberculatum*. 1 – Mature cleistothecia on hair bait × 5; 2 – Part of colony with young cleistothecia × 25; 3 – Cleistothecium initial × 137; 4 – Young cleistothecium × 137; 5 – Smoth young aleuriospore on short stalk × 137; 6 – Mature tuberculate aleuriospores × 400.

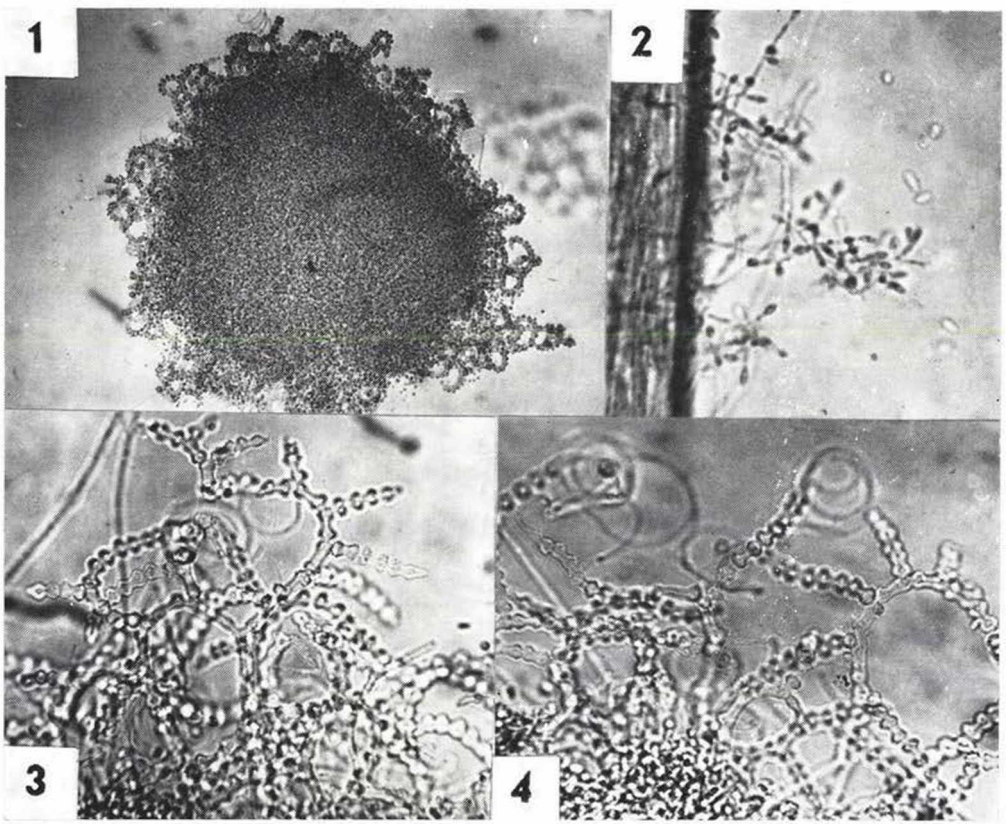

Fig. 3. *Arthroderma curreyi*. 1 – Mature cleistothecium × 110; 2 – Aleuriospores × 500, 3 – Peridial hyphae × 300; 4 – Peridial hyphae with coiled appendages × 300.